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Results of inoculation of beech (*Fagus sylvatica* L.) and oak (*Quercus* sp.) stumps with *Pleurotus ostreatus* (Jacq.: Fr.) Kumm.

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Abstract

The article presents the results of inoculation of beech and oak stumps with a biological preparation of oyster fungus (*Pleurotus ostreatus*) mycelium. The inoculum was grown on a sawdust substrate at Warsaw University of Life Sciences Department of Mycology and Forest Phytopathology. The experiment was set up at the Forest Experimental Station in Rogów. Stumps were inoculated: *i)* immediately after tree felling or *ii)* at approximately 5 and 12 months after felling. A total of 60 beech stumps and 50 oak stumps were inoculated in the years 2002–2004. The infected stumps were then inspected for the presence of the mycelium and fruiting bodies of *P. ostreatus*. The presence of fruiting bodies of other wood-decaying fungi was also noted. The extent of rotting was determined macroscopically, with the causal agent being confirmed on the basis of isolation and identification of mycelium from fragments of colonized wood obtained from rooted-out stumps. Oyster fungus mycelium was found to colonise beech stumps much more effectively than oak stumps. Stumps inoculated immediately after, or five months after felling were more effectively colonized by the mycelium than those inoculated after one year.

Key words

Artificial inoculation, *Fagus sylvatica*, *Quercus* sp., *Pleurotus ostreatus*, oyster fungus, stump decay

Introduction

Stumps left following tree felling are a permanent element of managed forests. Stumps may take several dozen years to mineralize, an essential component of which process is the activity of fungi, the only organisms capable of reducing wood to its initial constituents. Stumps become the site of competition of various organisms for food base. The numerous stump-colonising fungi include pathogenic species, such as *Armillaria* spp., which are dangerous parasites of forest trees.

In recent years, areas across Poland have been reporting increased damage to broadleaved stands attributable to fungi of the genus *Armillaria* (Rykowski 1990, Oszako 1997, Oszako and Delatour 2000, Sierota 2001, Szczepkowski 2001, Szczepkowski and Tarasiuk 2005, 2006, Tarasiuk and Szczepkowski 2006). *Armillaria* fungi contribute to dieback and excessive thinning in weakened stands of beech and oak. *Armillaria* root rot is difficult to control, as it is ubiquitous in the soil and inaccessible within the tree. Concomitant use of a number of methods of silviculture and forest protection is recommended in order to effectively limit the damage. A possible control measure can be the use of a biological method based on competitive or antagonistic organisms (Rykowski 1990, Sierota 1993, 1997, Butin 1995, Fox 2000, Żółciak 2002, 2005a, Oszako 2005). At the saprotrophic stage in their development, *Armillaria* fungi invade the dead wood of root and stumps remaining after a tending, sanitation or clear cutting. *Armillaria* control can be accomplished at this stage by inoculation of stumps with biological preparations containing appropriate wood-destroying fungi. The aim of this technique is to eliminate the food base of the pathogenic fungus, and thus reducing its infection potential by suppressing the production of mycelia, rhizomorphs and fruit bodies.

Various fungal species are being tested as promoters of stump decomposition. In Poland, studies on the inoculation of stumps with antagonistic fungi in order to control *Armillaria* root rot have been carried out by Orłoś (1957), Sierota (1993), Łakomy (1998, 2005a,b) Łakomy and Dux (1998), and Żółciak (2002, 2005b).

The aim of the present study was to explore the possibilities of using a biological preparation containing oyster fungus mycelium (*Pleurotus ostreatus*) for inoculation of beech and oak stumps at different time points after tree felling. The effects of the inoculation were assessed by examining the presence of oyster fungus mycelium and fruiting bodies or the extent of wood decay caused by the fungus. The quantitative and qualitative composition of lignicolous macrofungi found in the stumps were also recorded.

Materials and methods

A biological preparation containing oyster fungus (*Pleurotus ostreatus*) mycelium on a sawdust substrate was grown at the laboratory of the Department of Mycology and Forest Phytopathology, Warsaw University of Life Sciences. Beech and birch sawdust was enriched with crushed wheat grain (20% crushed wheat and 80% dry sawdust), soaked in distilled water, placed in polypropylene bags and autoclaved at 121°C for two hours. The cooled substrate was inoculated with oyster fungus mycelium and incubated at a temperature of 22°C until the substrate was visibly overgrown with the mycelium, which occurred after ca 12 weeks. The biological preparation was subsequently used to infect stumps left after clear cutting in forest stands belonging to the Forest Experimental Station in Rogów. The experiment was set up in five fresh mixed broadleaved forest sites, of which three were located in the Lipce Reymontowskie Forest Range (sections 13b,c and 15a) and the other two in the Strzelna Forest Range (sections 137a and 149a). Symptoms of *Armillaria* root rot had been confirmed in all study sites before setting up the experiment.

Prior to inoculation, an approximately 5 cm thick disk, or a half-disk of wood was cut off from the stump and 1 to 4 parallel or crossed cuts were made in it. Such cuts were not made in the thinnest stumps. The inoculum was then spread out over the entire upper surface of the stump, covered with the disk and fixed in place with a nail. The inoculation of stumps took place in the autumn of 2002 and in the spring of 2003 and 2004. A total of 60 beech stumps and 50 oak stumps were inoculated. A few stumps were left non-infected at each site to serve as controls.

The effectiveness of the inoculation was evaluated at approximately 6-month intervals (in spring and autumn). The spread of oyster fungus mycelium was assessed together with the presence of fruit bodies of the fungus and the abundance of other fungal species in the inoculated and control stumps. Trees located near the study sites were also inspected for the presence of the fruit bodies of oyster fungus.

In the spring of 2005, three beech stumps and three oak stumps were dug up, cleaved and separated into approximately 10 cm thick slices representing three layers (upper, middle and lower). The extent of rotting was determined macroscopically in the laboratory, and the causal agent (*Pleurotus ostreatus*) was identified by isolating a pure culture from the wood fragments. The fragments were cleaned and dried, and disinfected in ethyl alcohol. Next, 24 fragments were obtained from each of the layers and placed on a malt-agar medium. Thus a total of 72 fragments of wood were obtained and cultured from every stump inoculated in 2002 and 2003 (3 layers times 24 fragments), and 48 fragments were obtained from stumps inoculated in 2004 (from the upper and middle layers only).

■ Results

Oyster fungus mycelium was found to be a more effective coloniser of beech stumps compared to oak ones. Stumps inoculated in the spring immediately after tree felling or 5 months after felling bore more developed oyster fungus mycelium and a larger number of fruit bodies compared to stumps infected one year after felling in the autumn. Spring-inoculated stumps developed fruit bodies after only 5–6 months, while autumn-inoculated ones developed fruit bodies after as many as 12 months. The highest proportion (ca 80%) of beech stumps bearing fruit bodies of the oyster fungus was recorded in the autumn of 2004, 18 months after inoculation, which took place 6 months after tree felling (Tab. 1).

During that inspection, a maximum number of 65 fruit bodies 2 kg of total weight was collected from a beech stump 35 cm in diameter (Fig. 1). Fruit bodies of the oyster fungus were much less frequent on oak stumps, with only 7 out of 50 inoculated stumps (14%) demonstrating the presence of fruit bodies, which also appeared in much smaller numbers.

After 12 month follow-up, a beech stump (inoculated immediately after felling) was found to be invaded by oyster fungus mycelium to a depth of ca 10 cm. Two years after inoculation of another beech stump (inoculated at 5 months), *P. ostreatus* wood rot was seen to a depth of ca 30 cm from the stump surface (Fig. 2), confirmed by the successful isolation of a pure culture from different layers.

FIG. 1. A beech stump inoculated with *P. ostreatus* mycelium (18 months after treatment) with a record number of fruit bodies (photo by J. Piętka)

FIG. 2. A cleft beech stump (24 months after treatment) with visible oyster fungus mycelium under the bark and wood decay to a depth of about 30 cm (stump was inoculated 5 months after felling) (photo by J. Piętka)

TABLE 1
Numbers of beech stumps bearing *Pleurotus ostreatus* fruiting bodies in the years 2003–2006

Time of inoculation	Number of inoculated stumps	Age of stumps	Year of inspection							
			2003 *	2003 **	2004 *	2004 **	2005 *	2005 **	2006 *	2006 **
2002 **	20	12 months	0	13	0	8	2	4	1	0
2003 *	35	5 months	-	8	2	24	5	10	1	6
2004 *	5	fresh	-	-	-	1	0	3	1	2

* – spring
** – autumn

Oak stumps demonstrated colonisation by oyster fungus mycelium to a depth of ca 10 cm, mostly in sapwood. Pure cultures of *P. ostreatus* could not be isolated from stumps of both species inoculated at approximately 12 months after felling, 30 months after the treatment (Tab. 2).

TABLE 2
Numbers of pure culture isolates of *Pleurotus ostreatus* obtained from wood fragments of oak and beech stumps in 2005

Tree species	Diameter of stump (cm)	Date of stump inoculation	Months after inoculation	Number of pure culture isolates of *Pleurotus ostreatus* in individual stump layers		
				upper	middle	lower
Fagus sylvatica	40	2002**	30	0	0	0
	38	2003*	24	11	7	3
	30	2004*	12	16	0	-
Quercus sp.	37	2002**	30	0	0	0
	30	2003*	24	3	0	0
	38	2004*	12	5	0	-

* – spring
** – autumn

Fruit bodies of a total of 19 fungal species were recorded on inoculated beech stumps (Tab. 3), compared to 16 species on oak stumps (Tab. 4). Beech stumps were most frequently colonised by *Bjerkandera adusta* and *Trametes versicolor*, while oak stumps most often supported *Stereum hirsutum*.

TABLE 3

List of the fungi collected on beech stumps inoculated with *Pleurotus ostreatus* mycelium and their respective abundances during the entire investigation period

Species of fungus	Abundance
Armillaria sp.	+
Ascocoryne sarcoides (Jacy.) J. W. Groves & D. E. Wilson	+ +
Bjerkandera adusta (Willd.: Fr.) P. Karst.	+ + +
Calocera cornea (Batsch: Fr.) Fr.	+
Chondrostereum purpureum (Schum.: Fr.) Pouzar	+ +
Exidia plana (Wiggers) Donk	+
Ganoderma applanatum (Pers.) Pat.	+
Nectria cinnabarina (Tode) Fr.	+
Phlebia tremellosa (Schrad.: Fr.) Nakasone & Burds.	+ +
Pluteus atricapillus (Batsch) Fayod	+
Polyporus brumalis (Pers.) Fr.	+
Schizophyllum commune Fr.: Fr.	+
Trametes gibbosa (Pers.: Fr.) Fr.	+
Trametes hirsuta (Wulf.: Fr.) Pilát	+ +
Trametes versicolor (L.: Fr.) Pilát	+ + +
Ustulina deusta (Hoffm.) Lind	+
Xerula radicata (Relh.: Fr.) Dörfelt	+
Xylaria hypoxylon (L.) Grev.	+ +
Xylaria polymorpha (Pers.) Grev.	+

Explanations: + + + – abundant, + + – numerous, + – rare

TABLE 4
List of the fungi collected on oak stumps inoculated with *Pleurotus ostreatus* mycelium and their respective abundances during the entire investigation period

Species of fungus	Abundance
Armillaria sp.	+
Ascocoryne sarcoides (Jacy.) J. W. Groves & D. E. Wilson	+ +
Bjerkandera adusta (Willd.: Fr.) P. Karst.	+ +
Calocera cornea (Batsch: Fr.) Fr.	+
Daedalea quercina (L.: Fr.) Pers.	+
Hymenochaete rubiginosa (Schrad.: Fr.) Lév.	+
Lenzites betulinus (L.: Fr.) Fr.	+ +
Panellus stypticus (Bull.: Fr.) P. Karst.	+
Pluteus atricapillus (Batsch) Fayod	+
Psilocybe fascicularis (Huds.: Fr.) Noordel.	+ +
Psilocybe lateritia (Schaeff.: Fr.) Noordel.	+ +
Schizophyllum commune Fr.: Fr.	+
Stereum hirsutum (Willd.: Fr.) Gray	+ + +
Trametes hirsuta (Wulf.: Fr.) Pilát	+
Trametes versicolor (L.: Fr.) Pilát	+ +
Xylaria hypoxylon (Pers.) Grev.	+ +

Explanations: + + + – abundant, + + – numerous, + – rare

Fruit bodies of *Armillaria* sp. were found every year during autumn inspections in a few oyster fungus-inoculated stumps of beech and oak trees. All those stumps had been inoculated in the autumn of 2002, about 12 months after tree felling. Stumps of trees felled in winter and inoculated in the following spring bore some fruit bodies of *Armillaria* only in the second year of follow-up (two stumps) (Fig. 3).

To date, i.e. during the four years of the experiment, no fruit bodies of *P. ostreatus* have been found on the control stumps (not inoculated) or on living trees growing near the study sites.

FIG. 3. The competition for food base. Fruit bodies of *Armillaria* sp. (A) and *Pleurotus ostreatus* (B) growing out of the beech stump inoculated with oyster fungus mycelium (photo by A. Szczepkowski)

■ Discussion

The oyster fungus isolate used in the experiment demonstrated good ability to decompose broadleaved wood, with average mass loss of beech wood samples of 20.6% following 4 months' decomposition under laboratory conditions, compared to an average loss of 6.7% in oak (hardwood) samples.

Beech wood has low natural resistance to wood-decomposing fungi and therefore is readily invaded by a number of fungal species, including the oyster fungus. According to Kodrik (2001), the oyster fungus causes rapid decomposition of the wood of beech stumps. The degree of decomposition was approximately 50% four years after artificial oyster fungus infection, increasing to 90% after 6 years.

The oyster fungus usually grows as a saprotroph on dead wood or as a wound parasite or it may attack weakened trees (Butin 1995, Mańka 2005). It can be a threat to living trees, especially weakened or damaged individuals. Accordingly, future studies should concentrate on selecting and using forms of the oyster fungus that do not produce spores but are also characterised by good wood-decomposing ability. Non-sporificating forms of the fungus have become available in recent years (Gapiński et al. 2001). Kodrik (2001), in his study of stands where stumps were inoculated with oyster fungus mycelium, did not find its fruit bodies on living trees, even on artificially injured stems. The limited spread of the oyster fungus via spores may be related to the fact that the fruiting bodies of this species are edible, very tasty and can be collected by mushroom pickers.

The results of our experiment clearly show more effective colonisation of beech stumps by the oyster fungus compared to oak stumps. Importantly, the inoculated oak stumps were located in a large felled area subject to considerable exposure to sunlight, while the beech stumps were situated in a smaller felled area and within stands subject to group felling. This difference certainly had an impact on moisture levels in the oak stumps, which could have been a decisive factor in promoting successful inoculation. It should also be noted that oak hardwood is resistant to wood-destroying fungi. More effective decomposition of beech stumps was also revealed in a study by Żółciak (2002), in which inoculation was performed, among others, in beech and oak stumps obtained following a thinning. Łakomy (2005a) reported effective oyster fungus mycelium colonisation of both beech and oak stumps. Two years after inoculation, the stumps (beech, oak, birch) were overgrown with the mycelium to a depth of about 30 cm, a finding similar to the results of our experiment.

In our study, younger stumps inoculated in the spring presented better developed oyster fungus mycelium than older stumps inoculated in autumn. Fruit bodies could already be seen on spring-inoculated stumps 6 months after inoculation while autumn-inoculated stumps demonstrated fruit bodies only after 12 months. The spring months (April, May) are considered as the best time for artificial infection of stumps left after winter felling (Gapiński et al. 2001, Kodrik 2001).

The inoculated beech and oak stumps were found to contain fruit bodies of a total of 26 fungal species. Most of the fungi were common saprotrophs whose presence can indirectly affect stand health. Stumps inoculated 12 months after felling (beech and oak) supported the greatest variety of fungi, while those inoculated immediately after felling bore the lowest number of fungal species. Saprotrophic species developing on stumps must compete with pathogens for nutrients, which reduces their infective potential. The absence of pure cultures of the oyster fungus in beech and oak stumps inoculated 12 months after felling was probably due to earlier colonisation of these stumps by other fungi.

The success of the treatment was also dependent on human presence in the study sites as approximately 10% of the inoculated stumps in each subgroup were damaged (discs torn off).

■ Conclusions

1. Beech stumps were colonised more effectively by *Pleurotus ostreatus* mycelium than oak stumps.
2. Stumps inoculated immediately or five months after felling were more effectively colonised by oyster fungus mycelium than stumps infected one year after felling.

3. Spring inoculation of stumps left after felling in winter proved more effective than inoculation in autumn.
4. During four years of observation, stumps of beech and oak inoculated with oyster fungus mycelium were found to support a total of 26 species of fungi.

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■ Streszczenie (Summary)

Wyniki szczepienia pniaków bukowych (*Fagus sylvatica* L.) i dębowych (*Quercus* sp.) grzybnią boczniaka ostrygowatego (*Pleurotus ostreatus* [Jacq.: Fr.] Kumm.)

Zbadano możliwości wykorzystania boczniaka ostrygowatego (*Pleurotus ostreatus*) do szczepienia pniaków bukowych i dębowych w celu ograniczania bazy pokarmowej opieniek (*Armillaria* spp.). Biopreparat na podłożu trocinowym przygotowano w Zakładzie Mikologii i Fitopatologii Leśnej SGGW w Warszawie. Doświadczenie założono na 5 powierzchniach, na siedlisku LMśw, w Nadleśnictwie Rogów. Na wszystkich powierzchniach przed założeniem doświadczenia obserwowano występowanie objawów opieńkowej zgnilizny korzeni. Inokulowano pniaki powstałe zaraz po ścięciu drzew oraz ok. 5- i 12-miesięczne. W sumie, w latach 2002–2004, zaszczepiono 60 pniaków bukowych i 50 dębowych. Na zainfekowanych pniakach oceniano występowanie grzybni i owocników boczniaka ostrygowatego oraz obecność innych gatunków grzybów wielkoowocnikowych. Makroskopowo określano zasięg widocznej zgnilizny i potwierdzano sprawcę (*Pleurotus ostreatus*) poprzez izolację i identyfikację grzybni z fragmentów drewna wykarczowanych trzech pniaków bukowych i trzech dębowych. Stwierdzono, że grzybnia boczniaka ostrygowatego znacznie skuteczniej kolonizowała pniaki bukowe niż dębowe. Pniaki powstałe zaraz po ścięciu drzewa oraz 5-miesięczne były efektywniej zasiedlane przez grzybnię boczniaka niż pniaki inokulowane po roku od momentu ich powstania. W trakcie trwania doświadczenia na szczepionych pniakach bukowych odnotowano owocniki 19 gatunków grzybów wielkoowocnikowych, a na pniakach dębowych 16 gatunków. Pniaki bukowe najliczniej były kolonizowane przez *Bjerkandera adusta* i *Trametes versicolor*, natomiast pniaki dębowe przez *Stereum hirsutum*.

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